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(56) Documents Cited
GB 2254340 A EP 0577026 A2
WPI Abstract Accession Number 96-459414 and JP08229110 WPI Abstract Accession Number 96-015617 and JP07289854 WPI Abstract Accession Number 95-018416 and JP06304454

(58) Field of Search
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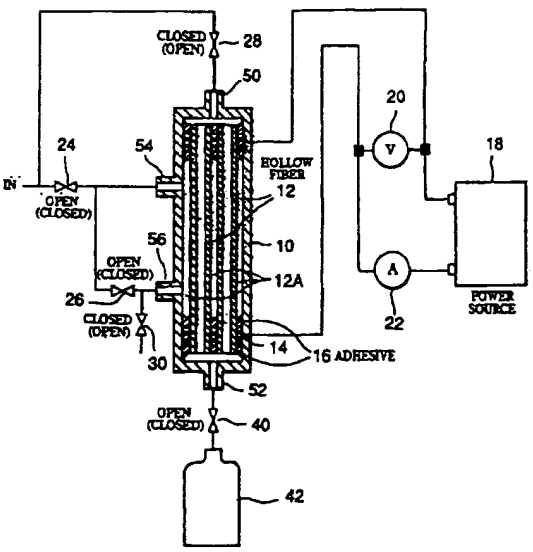
Online: WPI,PAJ

(54) Abstract Title
Sterilizing system and system for recovering genes

(57) A sterilizing system comprises an electrode consisting of a metal membrane and a porous membrane, where the porous membrane coats or is coated with the metal membrane, and means for supplying electricity to the metal membrane so as to kill microorganisms by electrification. The porous membrane may be a porous resin, sintered metal or a hollow ceramic tube and preferably consists of hollow fibres formed into a flat membrane. In use, a solution containing microorganisms into introduced into the chamber containing the electrode and the microorganisms are preferably trapped in the porous membrane while electricity is supplied to the metal membrane. The microorganisms treated by this system include cryptosporidium, pseudomonas aeruginosa, Legionella pneumophilia and E. Coli. The system may be used for recovering genes whereby destruction of E. Coli cells using the electrode allows genes to be selectively extracted from the cellular contents thus produced.

FIG.7



GB 2 329 633 A

FIG.1

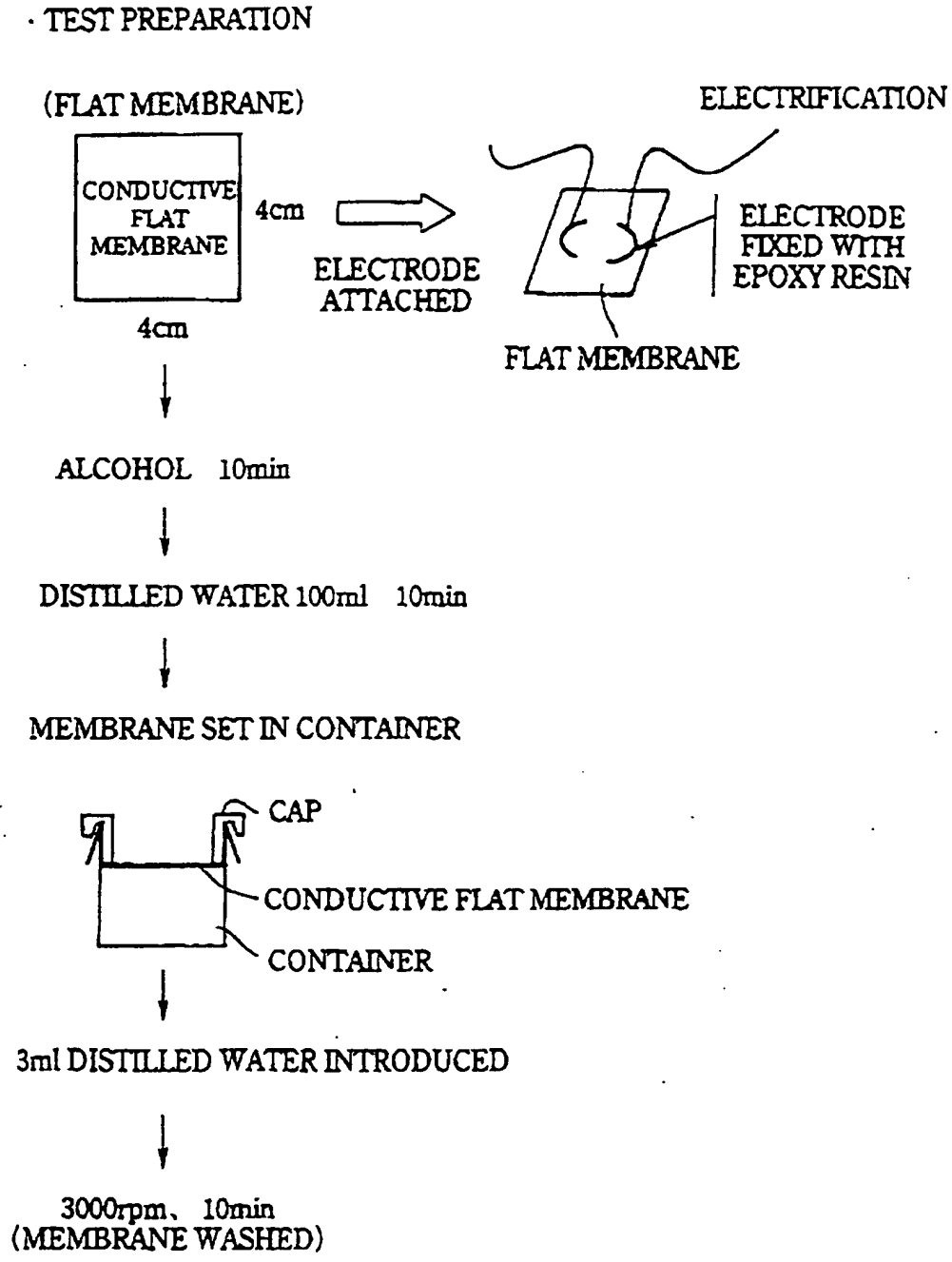

· TEST PREPARATION
(FLAT MEMBRANE)
CONDUCTIVE FLAT MEMBRANE
4cm
4cm
ELECTRODE ATTACHED
ELECTRIFICATION
ELECTRODE FIXED WITH EPOXY RESIN
FLAT MEMBRANE
ALCOHOL 10min
DISTILLED WATER 100ml 10min
MEMBRANE SET IN CONTAINER
CAP
CONDUCTIVE FLAT MEMBRANE
CONTAINER
3ml DISTILLED WATER INTRODUCED
3000rpm、10min
(MEMBRANE WASHED)

FIG.2

· D.C.300mA

INITIAL CELL COUNT (CFU/ml) 1.05×10^{9}

	CELL COUNT IN FILTRATE	CAPTURED CELL COUNT	RECOVERY CELL COUNT
no current	2.27×10^{7}	1.03×10^{9}	1.30×10^{8}
D.C.300mA	6.19×10^{7}	9.88×10^{8}	3.33×10^{7}

· D.C.500mA

INITIAL CELL COUNT (CFU/ml) 7.01×10^{8}

	CELL COUNT IN FILTRATE	CAPTURED CELL COUNT	RECOVERY CELL COUNT
no current	1.86×10^{7}	6.82×10^{8}	4.58×10^{7}
D.C.500mA	3.85×10^{8}	6.97×10^{8}	1.73×10^{7}

· D.C.750mA

INITIAL CELL COUNT (CFU/ml) 9.55×10^{8}

	CELL COUNT IN FILTRATE	CAPTURED CELL COUNT	RECOVERY CELL COUNT
no current	7.93×10^{7}	8.76×10^{8}	1.83×10^{7}
D.C.750mA	5.19×10^{7}	9.03×10^{8}	5.46×10^{6}

· D.C.1A

INITIAL CELL COUNT (CFU/ml) 6.74×10^{8}

	CELL COUNT IN FILTRATE	CAPTURED CELL COUNT	RECOVERY CELL COUNT
no current	9.00×10^{8}	6.65×10^{8}	2.13×10^{7}
D.C.1A	1.88×10^{7}	6.55×10^{8}	6.30×10^{3} (SIGNIFICANT EFFECT)

FIG.3

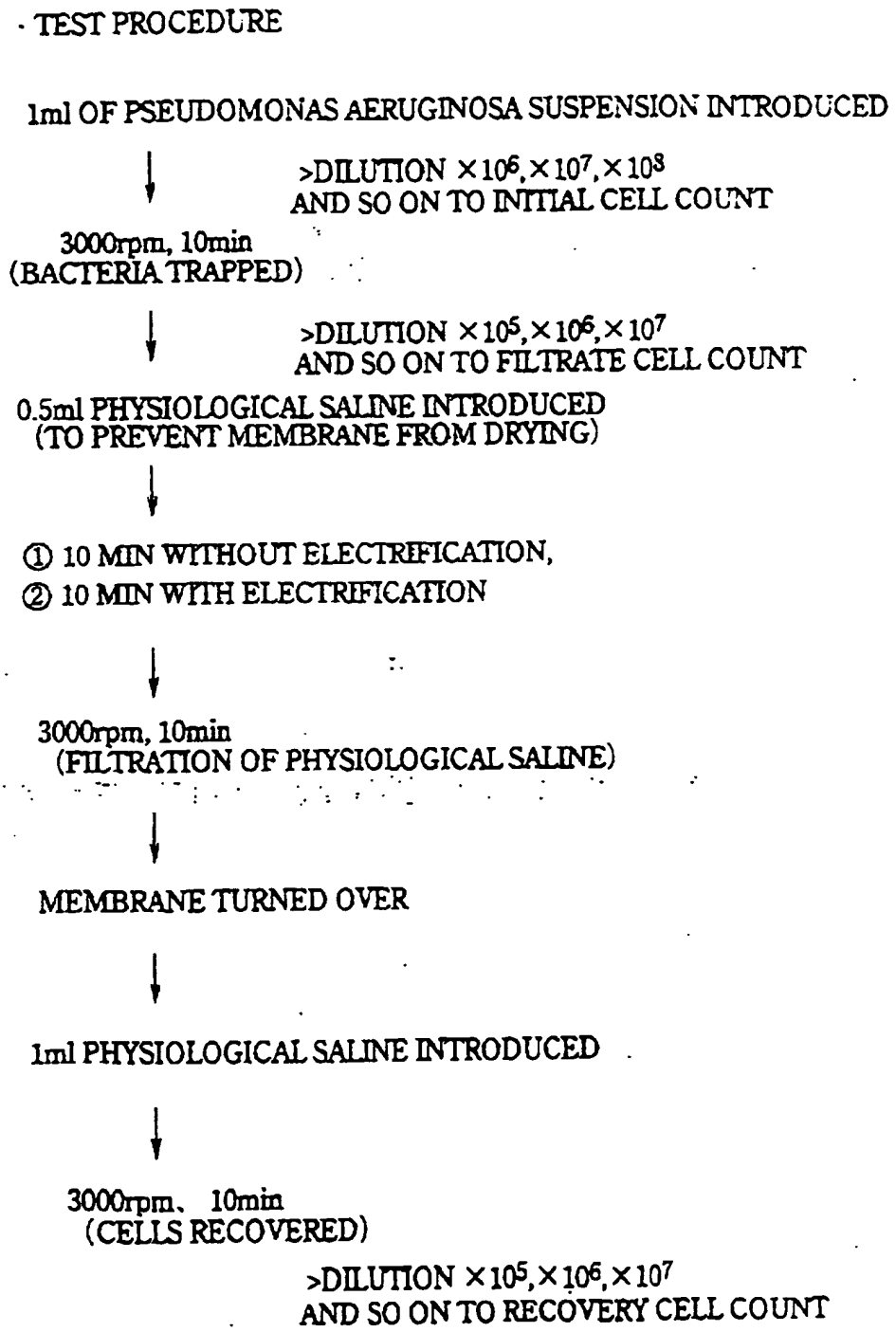

· TEST PROCEDURE
1ml OF PSEUDOMONAS AERUGINOSA SUSPENSION INTRODUCED
>DILUTION ×10⁶,×10⁷,×10⁸
AND SO ON TO INITIAL CELL COUNT
3000rpm, 10min
(BACTERIA TRAPPED)
>DILUTION ×10⁵,×10⁶,×10⁷
AND SO ON TO FILTRATE CELL COUNT
0.5ml PHYSIOLOGICAL SALINE INTRODUCED
(TO PREVENT MEMBRANE FROM DRYING)
① 10 MIN WITHOUT ELECTRIFICATION,
② 10 MIN WITH ELECTRIFICATION
3000rpm, 10min
(FILTRATION OF PHYSIOLOGICAL SALINE)
MEMBRANE TURNED OVER
1ml PHYSIOLOGICAL SALINE INTRODUCED
3000rpm、10min
(CELLS RECOVERED)
>DILUTION ×10⁵,×10⁶,×10⁷
AND SO ON TO RECOVERY CELL COUNT

FIG.4

CELL COUNT OF SOLUTION TRAPPED IN CONDUCTIVE MEMBRANE	CELL COUNT RECOVERED FROM ELECTRIFIED MEMBRANE	CELL COUNT RECOVERED FROM NON-ELECTRIFIED MEMBRANE
1.7×10^{12}cfu/ml	2.2×10^{7}cfu/ml	3.2×10^{9}cfu/ml

FIG.5

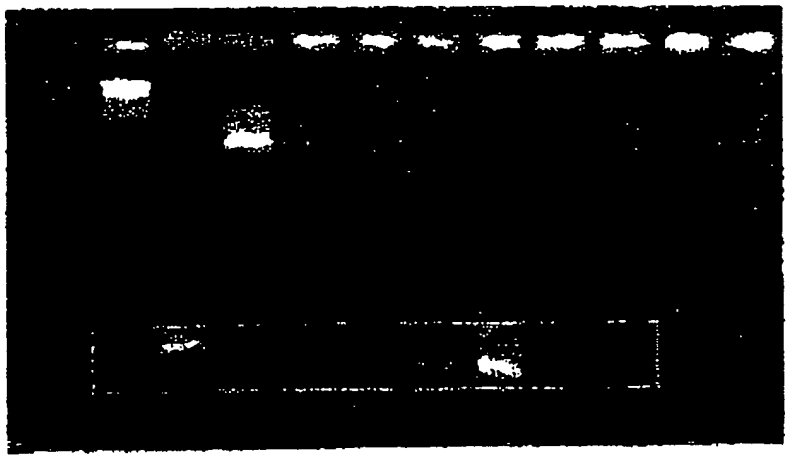


FIG.6

COMPARISON OF TIME NEEDED FOR RECOVERY USING CONDUCTIVE HOLLOW FIBER MEMBRANE AND TIME NEEDED FOR RECOVERY USING CONVENTIONAL METHOD

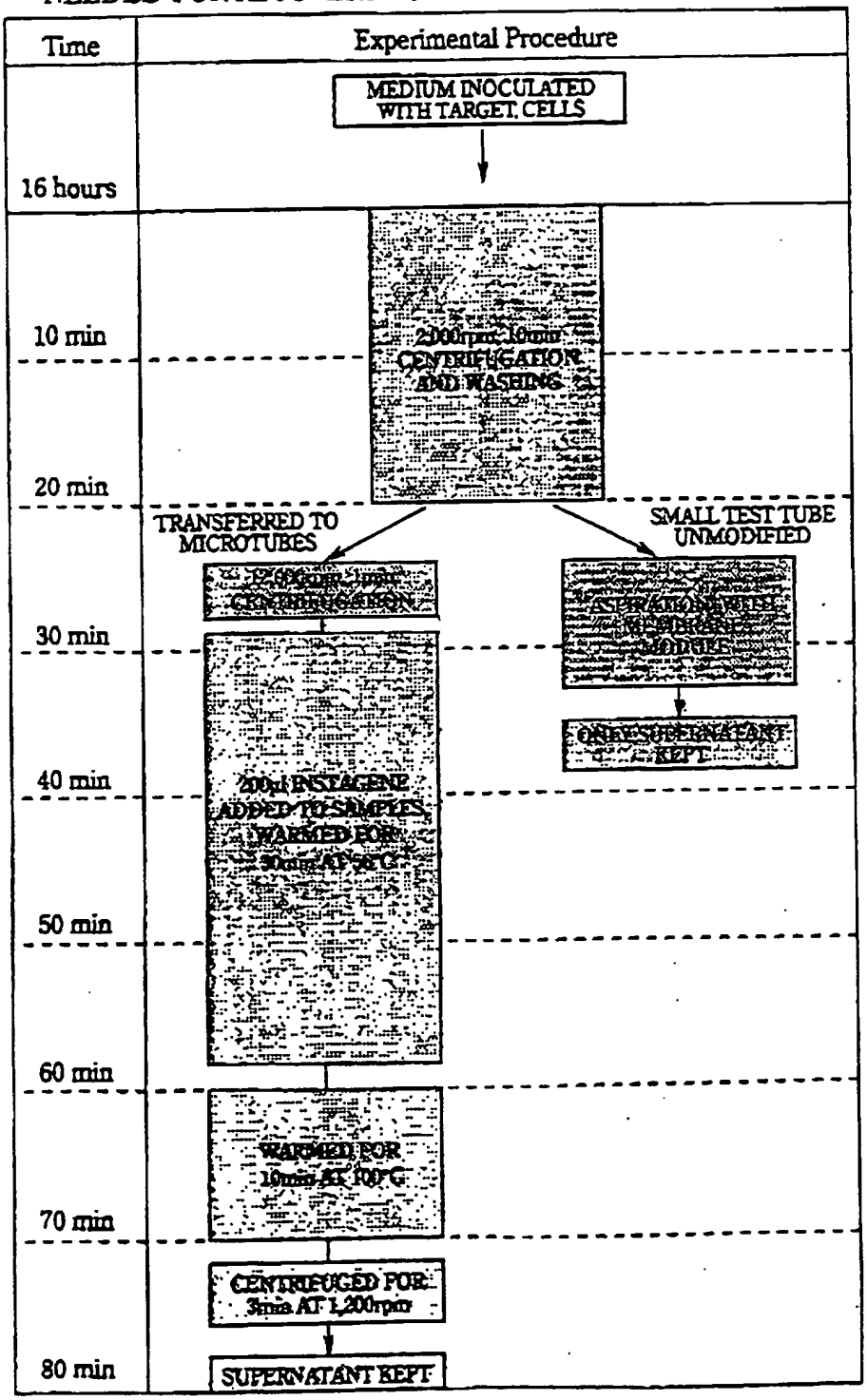
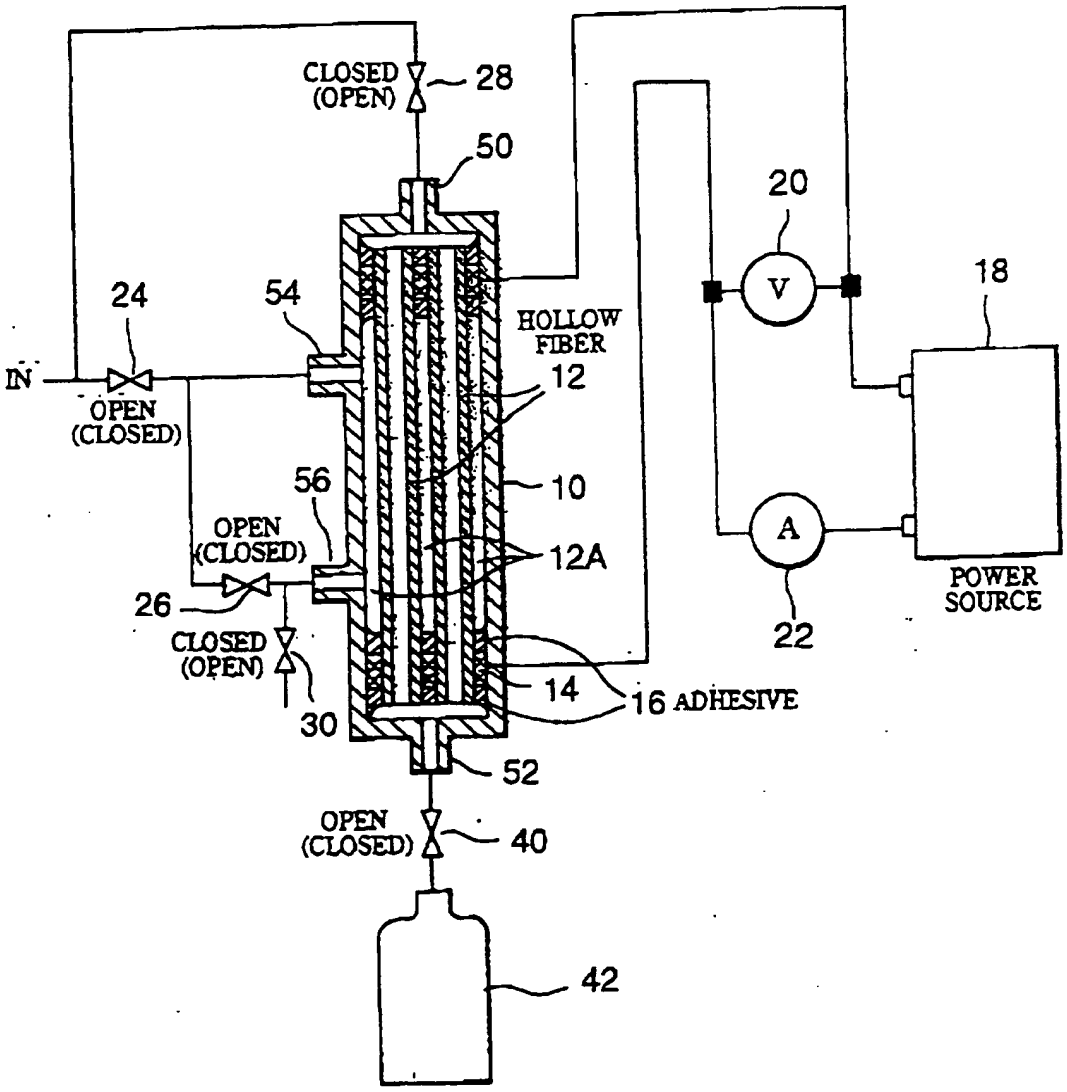


FIG.7


CLOSED
(OPEN)
28
50
20
V
18
24
54
HOLLOW
FIBER
IN
OPEN
(CLOSED)
12
56
10
OPEN
(CLOSED)
12A
A
POWER
SOURCE
26
CLOSED
(OPEN)
22
14
16 ADHESIVE
30
52
OPEN
(CLOSED)
40
42

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STERILIZING SYSTEM, AND SYSTEM FOR RECOVERING GENES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a sterilizing system, and a system for recovering genes from specific microorganisms.

2. Description of the Related Art

There has been much discussion recently on the adverse effects of pathogenic microorganisms. A first problem is the presence of microorganisms that contaminate human drinking water. Cryptosporidium, a protozoan that causes severe diarrhea and cramps in humans by infection through water and food, contaminates tap water sources and the like. Unfortunately, chorine-based sterilization is not effective against this protozoan. Chemicals with greater bactericidal effects than chlorine-based bactericides cannot be used in water sources.

A second problem is *Pseudomonas aeruginosa*, which causes hospital infection or opportunistic infection. *Pseudomonas aeruginosa* is also a source of infection in building reservoirs, with the risk of infecting humans through air conditioning or building drinking water. *Pseudomonas aeruginosa* is resistant to drugs.

A third problem is *Legionella pneumophila*, which is a source of infection in continuous household baths. *Legionella*

pneumophila is a cause of pneumonia. Despite talk of the use of ultraviolet rays to control the proliferation of this bacterium, it cannot be considered a satisfactory method of sterilization.

A fourth problem is pathogenic *E. coli*, particularly O-157, which causes food poisoning. The identification of the source of infection in food poisoning is indispensable to its prevention. Genetic analysis of O-157 is essential to identifying the route of infection. Japanese Unexamined Patent Application 9-178752 is an example of genetic analysis.

Chlorine-based bactericides are primarily used to prevent microorganisms which contaminate drinking water from adversely affecting humans. However, Cryptosporidium and the like are resistant to such bactericides.

Efforts have been made to kill *Legionella pneumophila* using ultraviolet rays to avoid the adverse effects which the use of chemicals can have on humans. However, a problem is that the aforementioned microorganisms cannot be sufficiently eradicated without the use of chemicals having considerable bactericidal effects.

Because the detection of bacteria by genetic analysis is a method of chemical analysis using enzymes in the conventional examples noted above, the system of analysis can become contaminated, resulting in poor analytical precision. Chemical treatments are also time-consuming.

In Japanese Unexamined Patent Application 9-37763, the present applicant proposed recovering intracellular genes by destroying the cells of microorganisms without the use of chemical components such as enzymes. However, this method is not considered a sufficiently quick and accurate method of genetic analysis for bacteria causing food poisoning, which require fast genetic identification, in order to rapidly ascertain the route of infection.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a system which allows the adverse effects of various pathogenic microorganisms to be eliminated by simple operations. Specifically, the invention is intended to provide an effective system of sterilization against microorganisms that contaminate drinking water, without the use of chemicals. The invention is also intended to provide a system for killing *Pseudomonas aeruginosa* and *Legionella pneumophila* without the use of chemicals. The invention is furthermore intended to provide a nucleic acid detecting system for the rapid and highly accurate genetic analysis of bacteria causing food poisoning.

To achieve these objects, the present invention is characterized by comprising electrification means having a metal membrane coated with a porous membrane, a power source for the electrification means, and supply means for supplying microorganisms to the electrification means, the microorganisms

being a source of contamination for drinking water, and the electrification means serving as an electrode to provide electricity from the power source to the microorganisms to kill the microorganisms.

The microorganisms are, in particular, the aforementioned Cryptosporidium, *Pseudomonas aeruginosa*, *Legionella pneumophila*, and pathogenic *Escherichia coli*.

The porous membrane comprises commercially available hollow fibers, flat membranes of such hollow fibers made into the form of a flat membrane, particularly those made of porous resin, ceramic hollow tubes, sintered tubes, and the like. The method disclosed in the aforementioned Japanese Unexamined Patent Application 9-37763 should be used to coat the metal with a porous resin. As noted in this report, the porous resin and metal should be chemically bonded. This allows more metal to be coated with the porous resin.

Electrification is essential in the present invention. The bactericidal effects are believed to be greater than those of electrodes consisting only of a metal sheet because the electrification takes place as the microorganisms are trapped in the porous material. There is a considerable difference in the rate of sterilization between cases with electrification and cases without electrification.

In this sterilization system, the membrane structure of the microorganisms is destroyed in the course of electrification,

allowing the nucleic acids which are the cellular contents to be obtained. The use of such nucleic acid for genetic analysis allows the genes of pathogenic *E. coli* to be identified, and the route of infection to thus be analyzed. In the conventional Japanese Unexamined Patent Application 9-178752 described earlier, enzymes were used to destroy the membrane structure of the cells, and a resulting problem was that a long time was needed for analysis when the system of analysis became contaminated.

Examples of configurations for such electrification include pulse electrification, direct current electrification, alternating current electrification, and impulse electrification. The examples below will show that direct current electrification is effective. It has also been indicated in Japanese Unexamined Patent Application 9-37763 that pulse electrification effectively destroys bacterial cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the steps for manufacturing the flat membrane coated with metal which is used in the present invention;

Fig. 2 is a diagram of properties, showing the results of sterilization against *Pseudomonas aeruginosa*;

Fig. 3 illustrates the steps in Example 1;

Fig. 4 is a diagram of properties, showing the test results for *Legionella pneumophila*;

Fig. 5 is a diagram of the properties of electrophoresis patterns based on the results of a test for recovering genes from pathogenic *E. coli;* and

Fig. 6 is an illustration of steps for comparing the time needed in a method for the chemical extraction of genes and a method of extraction using the system of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A sterilization system for *Pseudomonas aeruginosa* in a first embodiment of the present invention is described below.

System Structure:

A flat membrane comprising porous polypropylene resin (P120UA-04F, by Tonen Pirusu) was coated with silver using the method in section [0043] in Japanese Unexamined Patent Application 9-37763. As indicated in Fig. 1, this was cut to 4 cm^2, and lead wires were secured to the conductive flat membrane with low melting point solder and epoxy resin. The membrane was then washed for 10 min at 3000 rpm using 3 mL of distilled water.

The conductive flat membrane was washed for 10 min in ethyl alcohol and then for 10 min in 100 mL distilled water, it was then dried, and it was then affixed with a cap to the open end of a container. The conductive flat membrane had an electrical resistance of 0.3 Ω.

Microorganisms Used:

Sterilization tests were conducted using *Pseudomonas aeruginosa* and *Legionella pneumophila* serogroup 1. The *Legionella pneumophila* used in the test was isolated from cooling water in building air conditions, and was identified as serogroup 1 based on the biochemical description and serological analysis.

Method of Sterilization:

1 mL of bacterial solution adjusted to a constant cell count was placed on the conductive flat membrane, and the bacteria were trapped in the membrane by centrifugation. Physiological saline was then added in the form of drops to prevent drying, and 1 A of current was applied for 10 min. Following the conclusion of electrification, the membrane was turned over, 1 mL of physiological saline was placed on the reverse side, and the bacteria trapped in the membrane were forcibly removed from the membrane and recovered. As a control, membranes were prepared with only electrification, and the cells recovered from the membrane were counted.

Test Results:

The test results are given in Fig. 2. Fig. 3 shows the steps of the test procedure. The tables in Fig. 2 give the results of sterilization in terms of the level of current applied in each case. The expression "no current" means that only electrification was omitted after the bacterial cells had been trapped in the membrane, whereas the tables also give the various levels of electrification for those cases in which electrification

treatment was carried out. The initial cell count is the cell count of the bacterial solutions placed on the membranes, the cell count of the filtrate is the cell count after the solution had been passed through the membrane, and the captured cell count is the difference of the cell count of the filtrate from the initial cell count. The effects of electrification can be calculated by the recovery cell counts in the tables. The recovery cell counts are the cell counts of the solutions in which the cells in the membranes were forcibly removed by centrifugation after the cells had been trapped in the membranes, the membranes had then been turned over, and physiological saline had been placed on the reverse.

The *Pseudomonas aeruginosa* was adjusted in the following manner. Cells were pre-incubated overnight in ampules (3 mL). They were washed twice using a centrifuge. The cells were resuspended in 3 mL physiological saline to prepare *Pseudomonas aeruginosa* suspensions (10^8 to 10^9 CFU/mL).

The results in Fig. 2 show that the effects of electrification on *Pseudomonas aeruginosa* using 750 mA DC resulted only in about a 25% reduction compared to groups with no electrification, whereas 1A current reduced the cell count to as low as 0.03% compared to the groups with no electrification. These results make it clear that *Pseudomonas aeruginosa*, which cannot be readily killed with conventional antibiotics or a variety of other chemicals such as chemotherapeutic agents, can be killed in a short

period of time without using such chemicals. Electrification of at least 750 mA is thus desirable.

Embodiment 2

Sterilization tests against *Legionella pneumophila* were carried out by the same method as in Embodiment 1.

Fig. 4 shows the results of the sterilization tests. Based on the test results, only about 10^7 cells could be recovered even when the cells were forcibly recovered by centrifugation after 10^{17} cells had been trapped, so the flat conductive membranes were highly effective in trapping the cells. Electrification is effective against bacterial cells because of the high trapping properties of the conductive flat membranes. It may thus be seen that *Legionella pneumophila* could be killed by electrification treatment because the recovered cell count was further reduced to about 1/100 by electrification treatment. *Pseudomonas aeruginosa* and *Legionella pneumophila* were difficult to eradicate in the past, but such cells can be effectively killed with this system.

Embodiment 3

Genes were extracted from pathogenic *E. coli* (*Escherichia coli* serotype O-157) according to sections [0043] through [0058] in Japanese Unexamined Patent Application 9-37763.

The genes that had been obtained were then amplified by PCR using Progene by Techne. The resulting genes were

electrophoresed to obtain electrophoresis patterns. Fig. 5 shows the patterns.

In Fig. 5, ((1)) is a gene marker (gene for finding verotoxin). ((2)) is the target gene (verotoxin-producing gene). ((3)) is the gene pattern obtained by chemical extraction with 10^3 cell/mL (using "Instagene," a reagent for DNA purification and recovery by Bio-Rad). ((4)) is a 1/10 chemical extraction method (10^7 cell/mL). ((5)) is extraction without electrification. ((6)), ((7)), ((8)), and ((9)) are the results for genes extracted with electrification under conditions involving electrification of 100 mA, 100 mA, 300 mA, and 500 mA (10^3 cells/mL for both electrified and non-electrified). Bands that appear white in the white lines are for the valerotoxin gene.

The details of the method for chemical extraction are given below. (1) Samples were washed with phosphate-buffered physiological saline and concentrated. (2) The samples were resuspended in distilled water. (3) Instagene was added to the samples, and they were incubated for 30 minutes at 56°C. At this stage, the cell enzymes were destroyed and the genes aggregated. (4) The samples were heated for 8 min at 100°C to thermally denature the enzymes or other proteins and allow the genes to aggregate. (5) The material was thoroughly mixed to produce PCR samples.

As is clear in Fig. 5, no bands showed up in samples that were not electrified, whereas bands did show up in those that were electrified, confirming the isolation of the O-157 gene.

Patterns for contaminants showed up above and outside the bands (target gene) in the method of chemical extraction. This did not show up in the electrified samples. Genes could thus be extracted in a purer state by electrification than by chemical extraction.

Fig. 6 is an illustration of steps for comparing the time needed to recover genes in the method of gene extraction using electrification and the time needed to recover genes in the method of chemical extraction. The time needed to complete gene recovery in the former was about half the time needed in the latter. That is, the cell pre-incubation and cell harvest took about the same amount of time in both, but the cells were completely destroyed in about 12 min in the former, whereas more than an hour was needed in the latter.

There were also considerable differences between the two in terms of the time needed in the pre-incubation for gene extraction. That is, because the former is a method for the recovery and extraction of genes with high purity or high yields, the number of cells that are needed can be no more than 1/10 that of the latter. Samples with a cell count of about 10^9 cells/mL are needed for 16 hours of pre-incubation in the latter method. Because the former method may have 1/10 or less of that, the pre-incubation may be no more than 1 hour. That is, nearly 17 hours are needed from the pre-incubation of the cells to the extraction of the genes in the latter method, whereas no more than 2 hours are needed in the former method.

Ultimately, unlike the latter method of chemical extraction, the former method is a method of gene extraction that is simpler and faster, with higher purity and yields. Since fewer chemical components are used than in the latter method, there is that much less opportunity for contamination, allowing genes of higher purity to be obtained.

Although flat membranes were used in Embodiments 1 and 2 above, membranes of hollow fibers coated with metal may also be used.

The structure of the apparatus used to realize the system of the present invention is described below using Fig. 7. In Fig. 7, the symbol 10 indicates a container housing hollow fibers coated with metal. The symbol 12 indicates hollow fibers coated with metal. The symbol 12A indicates the space between the hollow fibers. Both ends of the hollow fibers are fixed to a metal support 14. The support serves as a contact when electricity is supplied to the metal coating of the hollow fibers. The symbol 16 is an adhesive for fixing the contact to the housing.

The symbol 18 is a power source. Electricity is supplied from the power source to the aforementioned contact. The symbol 20 is a voltmeter and the symbol 22 is an ampere meter. Openings for supplying the liquid to be treated in the container are provided at both ends of the container facing the aforementioned contacts. The openings are indicated by 50 and 52. Similar openings 54 and 56 are provided on the sides of the container. Openings 50 and 52 pass only through the two ends of the hollow

fibers. Openings 54 and 56 pass through only the space between the hollow fibers.

Tubes forming passages through which liquids flow are connected to these openings. The tube structure is depicted in Fig. 7. The part where the tubes intersect shows that the liquid flows in both directions in the intersecting part.

Symbols 24, 26, 28, 30, and 40 each indicate a valve. The valves are each placed in either an open or closed state. In the figure, "open" indicates that the corresponding valve is in an open state, allowing liquid to pass through. Conversely, "closed" indicates that the valve is in a closed state. A legend indicating whether a valve is open or closed appears to the side of the valve. In the figure, the legends that are not in parentheses indicate whether or not the valves are open or closed when the liquid containing the microorganisms is sent through the container for sterilization. The legends in parentheses indicate whether the valves are open or closed when the hollow fibers are washed following the conclusion of the sterilization treatment.

When a solution is to be sterilized, the solution that is to be treated is introduced from the location indicated by "IN" in the figure. The solution passes through valves 24 and 26 to openings 54 and 56, respectively. The solution supplied from the openings passes through the side walls of the hollow fibers into the interior of the hollow fibers, and then through the opening 52 and the valve 40 into a recovery container 42. During this

step, the solution comes into contact with the metal coating the hollow fibers. The microorganisms in the solution are electrified by the metal at this time. As a result, the undesirable microorganisms in the solution are killed or attenuated.

The porous material of the hollow fibers must be periodically washed because it becomes clogged. In such cases, purified water is introduced from IN as the valves are put into the stated indicated in parentheses. The purified water at this time passes through the valve 28, through the opening 50, from the top end of the hollow fibers, into the interior of the hollow fibers. Because the opening 52 is closed at this time, the purified water passes from inside the hollow fibers, through the side walls of the hollow fibers, and into the opening 56, and it is then discharged through the valve 30.

As described above, the present invention makes it possible to provide a system that allows the adverse effects caused by various pathogenic microorganisms to be eliminated by simple operations. That is, the invention provides a sterilization system that is effective against microorganisms contaminating drinking water without the use of chemicals. The invention also provides a system for killing *Pseudomonas aeruginosa* and *Legionella pneumophila* without the use of chemicals. The invention furthermore provides a nucleic acid detecting system for the rapid and highly accurate genetic analysis of bacteria causing food poisoning.

What Is Claimed Is:

1. A system comprising:

electrification means having a metal membrane coated with a porous membrane;

a power source for the electrification means;

supply means for supplying microorganisms to said electrification means, said microorganisms being a source of contamination for drinking water, and said electrification means serving as an electrode to provide electricity from said power source to the microorganisms to kill the microorganisms.

2. The system according to Claim 1, wherein said microorganisms are at least one of Cryptosporidium, *Pseudomonas aeruginosa*, *Legionella pneumophila*, and pathogenic *Escherichia coli*.

2. A system comprising:

electrification means having a metal membrane coated with a porous membrane;

a power source for the electrification means;

supply means for supplying microorganisms to said electrification means, said microorganisms being at least one bacterium from among *Pseudomonas aeruginosa*, *Legionella pneumophila*, and pathogenic *Escherichia coli*; and

said electrification means serving as an electrode to provide electricity from said power source to the microorganisms to kill the microorganisms.

4. The system according to Claim 1 or 3, wherein said electrification means provides direct current to said microorganisms.

5. The system according to Claim 1 or 3, wherein said electrification means provides electricity while said microorganisms are trapped in the porous membrane.

6. The system according to Claim 1 or 3, wherein said metal is chemically bonded to the porous resin constituting said porous membrane.

7. A system for recovering genes, comprising:

electrification means having a metal membrane coated with a porous membrane;

a power source for the electrification means; and

supply means for supplying pathogenic *E. coli* to said electrification means, said electrification means serving as an electrode to provide electricity from said power source to the microorganisms to destroy said *E. coli* cells, thereby allowing genes to be selectively extracted from the cellular contents thus produced.

8. A method for applying microorganisms which are a source of contamination in drinking water to the porous membrane with which the metal has been coated, and then providing electricity from the power source to said metal membrane, so as to kill said microorganisms.

9. The method according to Claim 8, wherein said microorganisms are at least one of Cryptosporidium, *Pseudomonas aeruginosa*, *Legionella pneumophila*, and pathogenic *Escherichia coli*.

10. The system according to Claim 7, wherein said porous membrane is a porous resin membrane.

11. The method according to Claim 8, wherein said porous membrane is a porous resin membrane.

The Patent Office

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Application No: GB 9820822.6 **Examiner:** Gavin Dale
Claims searched: 1-11 **Date of search:** 23 November 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): A5G (GAB); C1C (CJA, CKA, CLA, CRX, CSX, CTX)

Int Cl (Ed.6): A61L 2/02; C02F 1/46, 1/467; C12N 1/06; C25B 11/03

Other: Online: WPI, PAJ

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
Y	GB 2254340 A	(NIKKO KOGYO KK) See Fig 22, page 5 line 24 to page 6 line 3 & page 36 lines 16-22	8 at least
X	EP 0577026 A2	(NAGAURA) See column 3 lines 27-37 & column 4 lines 4-16	8 at least
Y	WPI Abstract Accession Number 96-459414 & JP08229110	(NIKKO KOGYO KK) See WPI abstract and Patent Abstract of Japan abstract	8 at least
Y	WPI Abstract Accession Number 96-015617 & JP07289854	(NIKKO KOGYO KK) See WPI abstract and Patent Abstract of Japan abstract	8 at least
Y	WPI Abstract Accession Number 95-018416 & JP06304454	(NIKKO KOGYO KK) See WPI abstract and Patent Abstract of Japan abstract	8 at least

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